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IDENTIFICATION OF A TREATMENT-RESISTANT, KETAMINE-SENSITIVE GENETIC
LINE IN THE CHICK ANXIETY-DEPRESSION MODEL

A Thesis

Presented in partial fulfillment of requirements

For the degree of Master of Arts in the department of Psychology

The University of Mississippi

by

STEPHEN W. WHITE

Spring 2014

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ABSTRACT

Approximately 60% of Major Depressive Disorder (MDD) patients do not respond to FDA-approved antidepressants. Introducing effective pharmacotherapies for this treatment-resistant population is hindered by the lack of pre-clinical screening assays that accurately model the clinical features of TRD. The purpose of this research was to screen representatives of different classes FDA-approved antidepressants and one novel antidepressant in two genetic lines of domestic fowl chicks that have previously been identified as stress-vulnerable and stress-resilient in the chick anxiety depression model. Separate groups of socially raised Black Australorp (stress-vulnerable) and Production Red (stress-resilient) chicks were administered the tricyclic antidepressant imipramine (0-20 mg/kg), the selective serotonin reuptake inhibitor (SSRI) fluoxetine (0-10 mg/kg), the tetracyclic antidepressant maprotiline (0-10 mg/kg), the glutamate receptor antagonist ketamine (0-15 mg/kg), or vehicle (physiological saline) and placed individually inside sound attenuating chambers for a 90-minute test period. The behavioral measure of distress vocalizations (DVocs) was recorded via custom designed software. Replication and validation of previous findings of stress sensitivity in the two genetic lines was measured by calculation of the onset of behavioral despair during the depression like phase (30-90 min). Verifying previous research, Black Australorps entered behavioral despair approximately 25% faster than Production Reds signifying the stress-vulnerability of the Black Australorp line. In the depression-like phase, Black Australorps were insensitive to imipramine and fluoxetine, but sensitive to ketamine, a finding that parallels the clinical picture of TRD.

Utilization of the Black Australorps genetic line in the chick anxiety-depression model may be a novel and lone preclinical screening to identify alternative mechanisms and promising leads for TRD.

DEDICATION

This thesis is dedicated in memoriam to my parents, James W. White and Martha M. White for their support in my decision to return to school and complete my undergraduate degree and to pursue graduate degrees.

LIST OF ABBREVIATIONS AND SYMBOLS

MDD	Major Depressive Disorder
DSM-IV-TR	Diagnostic and Statistics Manual-IV-Text Revision
TRD	Treatment Resistant Depression
FDA	Food and Drug Administration
TCA	Tricyclic Antidepressants
TeCA	Tetracyclic Antidepressants
SSRI	Selective Serotonin Reuptake Inhibitors
NMDA	N-methyl-D-aspartate
TST	Tail-Suspension Test
FST	Forced-Swim Test
CMS	Chronic Mild Stress
DVocs	Distress Vocalizations
BDNF	Brain Derived Neurotrophic Factor
IP	Intraperitoneal
ANOVA	Analysis of Variance
LSD	Least Significant Difference
n.s.	Non-Significant
mTOR	Mammalian Target of Rapamycin
NET	Norepinephrine Transporter

ACKNOWLEDGMENTS

I express my deepest appreciation to my advisor and mentor Dr. Kenneth J Sufka, my committee members, Drs. Karen Sabol and John Young.

In addition I would like to thank my fellow graduate students and the undergraduate research assistants that comprise the Psychopharmacology Lab, without whom I could not have collected data and completed this research.

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CHAPTER 1

INTRODUCTION

According to the National Institute of Mental Health, major depressive disorder (MDD), or major depression, is characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat, and enjoy pleasurable activities. Some people may experience only a single depressive episode within their lifetime, but more often a person may suffer from multiple depressive episodes. According to the Diagnostic and Statistics Manual-IV-text revision (DSM-IV-TR), five of the following nine criteria must be met in the same two week period to qualify someone as suffering from a major depressive episode: 1. depressed mood most of the day, nearly all day; 2. diminished interest or pleasure in all, or almost all activities most of the day nearly every day; 3. significant weight loss when not dieting or significant weight gain, or a decrease or increase in appetite nearly every day; 4. insomnia or hypersomnia nearly every day; 5. psychomotor retardation or agitation nearly every day; 6. fatigue or loss of energy nearly every day; 7. feelings of worthlessness or excessive or inappropriate guilt nearly every day; 8. diminished ability to think or concentrate, or indecisiveness, nearly every day; 9. recurrent thoughts of death other than fear of death, recurrent suicidal ideation, or a suicide attempt, or a specific plan for committing suicide (Diagnostic and Statistics Manual-IV-TR). A 2003 study using 9090 participants found that 16.2% of the participants suffered from an MDD episode during their lifetime and that 6.6% of the participants experienced a major depressive

episode during the 12 months before the study. If those sample numbers were extrapolated to the general population, the study suggests that between 32-35 million U.S. adults experience a major depressive episode in their lifetime and between 13-14 million U.S. adults experience at least one depressive episode during a 12-month period (Kessler et al, 2003). In a 2005 follow up study designed to estimate 12- month prevalence, severity, and comorbidity of DSM-IV anxiety, mood, impulse control, and substance abuse disorders found that 6.7% of the subjects experienced a major depressive episode in the preceding 12 months and 1.5% of the subjects suffered from a dysthymic episode during the same period, findings consistent with the 2003 study. The study found that anxiety disorders were more prevalent but that the severity of depressive episodes was more extreme (Kessler et al., 2005). The estimated economic cost of MDD in 2000, which includes the cost of treatment and loss of work productivity, was estimated at \$83.1 billion in the United States alone (Greenberg et al. 2003).

There are several forms of depression besides MDD, such as dysthymic disorder, minor depression, and seasonal affective disorder. The symptoms of these depressive disorders may not be as severe as the symptoms of MDD, but can interfere with normal functioning and inhibit a sense of well-being. In addition to these, another unique form of depression is treatment-resistant depression (TRD). As it is reported that upwards of 60% of MDD patients do not respond to currently approved antidepressants, a significant portion of MDD patients have residual or persistent symptoms despite adequate antidepressant therapy. Due to this low response rate, typical patient treatment involves a serial dosage escalation, drug rotation, or augmentation with other compounds. Compliant patients unresponsive to adequate dosing under adequate duration of at least two different classes of antidepressants are then qualified as having TRD. It follows, patients with TRD are more likely to suffer greater comorbidity with other

psychiatric syndromes, greater functional impairment due to longer illness duration, lower remission rates and greater risk of depressive symptom recurrence, including an increased rate of suicide attempts. (Nelsen and Dunner, 1995, Kornstein and Schneider, 2001). A 2010 study by Fostick et al. found a positive correlation between depression severity and economic impact (Fostick et al., 2010). Patients with more severe depression had increased direct costs, such as blood and imaging tests, physician visits, and hospitalization costs, as well as increased indirect costs, such as lower worker productivity and higher worker absenteeism. In a recent meta-analysis of the cost of MDD patient care from 2001 to 2009, it was found that the medical expenses for TRD patients was almost 30% higher than normal MDD patients (Olchanski et al., 2013). The severe and ongoing depressive symptomology along with the increased economic impact for TRD patients make finding effective treatments for this population paramount and this depends on the development, and utilization, of preclinical animal models of TRD.

CHAPTER 2

BACKGROUND

The goal of any treatment for a psychological disorder is the full remission of disorder symptomology defined as full patient recovery of psychological and social functioning without any residual disorder effects. For over 60 years pharmacotherapy for the treatment of MDD has targeted the monoaminergic system in effort to raise synaptic serotonin, norepinephrine, and dopamine levels. Current classes of antidepressants approved by the Food and Drug Administration (FDA) include tricyclic antidepressants (TCA's), such as imipramine, tetracyclic antidepressants (TeCA's), such as maprotiline, and selective serotonin reuptake inhibitors (SSRI's), such as fluoxetine. However, these drugs are not without their shortcomings. Firstly, it is reported that upwards of 40 to 60% of MDD patients do not respond (i.e. do not show depressive symptom alleviation) to these drugs (Paul, Skolnick, 2003; Trivedi, Rush, Wisniewski, 2006). Two studies of placebo controlled and double blind placebo controlled studies found that upwards of 50% of all patients treated with a single antidepressant failed to reach full symptom remission (Fava, Davidson, 1996; Golden et al., 2002). Further, in a more recent 2010 study, Iovieno et al., found that out of 576 MDD patients treated with the SSRI fluoxetine for 12 weeks, only 203, or 35%, met criteria for remission as measured using the Hamilton Depression Rating Scale. Of those 203 patients deemed to be in remission, over 90% had at least one residual depressive symptom with the median number of residual symptoms equaling four (Iovieno, N. et al., 2011). Secondly, even though these compounds do produce an

acute increase in synaptic monoamine levels, 3 to 6 weeks of drug administration is needed to produce adequate MDD symptom relief thus prolonging depressive symptomology (Paul, Skolnick, 2003; Trivedi, Rush, Wisniewski, 2006). The lack of efficacy of current antidepressants for MDD sufferers generally leads to a serial trial and error approach of prescribing antidepressant medication, which includes dose escalation, drug rotation, and/or antidepressant augmentation with other psychoactive drugs designed to enhance or facilitate the effects of antidepressants. At this point, patients who fail to respond to adequate dosing of adequate duration and at least two classes of antidepressants are considered to be treatment resistant and are diagnosed with TRD.

While typical antidepressants fail to provide MDD symptom relief in these patients, a number of clinical studies have shown that low doses (i.e. non-psychotomimetic) of ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist produces rapid, long-lasting effects in TRD patients (Zarate et al., 2006, Price et al., 2009). Although these current clinical tests of low doses of ketamine show positive results for alleviating MDD and, more importantly, TRD symptomology, the use of ketamine and the blockade of NMDA receptors are not without their dangers. Higher doses of ketamine have long been known to possess dissociative effects and has been used as a general pre-surgery anesthetic (Domino, E., Chodoff, P., Corssen, G., 1965, Corssen, G., Miyasaka, M., Domino E., 1968). However, at sub-anesthetic doses, ketamine possess abuse/addictive potential (Siegel, R., 1978). Furthermore, NMDA receptors play a key role in learning and memory (Morris, R., Anderson, E., Lynch, G., Baudry, M., 1986) and, as such, may not be a viable target for treating TRD, or MDD, as highlighted in recent research by Murrough et al. in 2013. The study examined the neurocognitive effects of ketamine in patients with TRD and found that after an intravenous infusion of 0.5 mg/kg dose of ketamine, patients

showed cognitive impairments as measured by scores on a battery of cognitive tests including intelligence quotient (IQ) tests and the MATRICS Consensus Cognitive Battery test (Murrough et al., 2013).. Despite its therapeutic effects in TRD, ketamine's side effect profile along with its mechanism of action (blockading the NMDA receptor) make it an unlikely long term candidate for clinical use. However, its efficacy in TRD is encouraging and requires further research in finding alternatives for alleviating TRD symptomology.

Furthering our understanding of the etiology, pathology, and treatment of psychological disorders relies on the use of animal models as simulations. Currently, rodent models, such as the tail-suspension test (TST) the forced-swim test (FST), the chronic mild stress test (CMS), and the social defeat paradigm are employed as simulations of major depressive disorder and as preclinical screens for effective antidepressants. A major validity issue in rodent simulation is the degree of their pharmacological sensitivity; in some instances, a model may be vulnerable to false positives where a drug shows efficacy in the animal model yet fails in clinical trials and/or it may be vulnerable to false negatives where a drug screens ineffective in the model yet would have benefited individuals with syndrome (for review Wilner, 1991). Furthermore, to date, no rodent model of depression can accurately present the clinical features of TRD. Some CMS models have shown insensitivity to a single class of antidepressants wherein one or two doses of an SSRI (fluoxetine or escitalopram respectively) failed to reverse anhedonia related behaviors in a small population of the test animals (Isingiri et al., 2010; Jayatissa et al., 2006). While these studies are of note, they fail to replicate the clinical picture of TRD in that only one class of FDA approved antidepressant was administered and shown to fail and this failure was only seen in a small number of test animals. In addition, and perhaps most importantly, they also failed to

show the efficacy of ketamine along with these drug probe failures simultaneously in one pre-clinical model.

As an alternative that addresses the concerns above, we have developed an anxiety-depression simulation/screening paradigm using an avian model. This chick paradigm appears to provide a more clinically relevant non-rodent based model of a neuropsychiatric syndrome and appears useful as a high-utility, dual-drug screen for anxiolytic and antidepressant compounds.

The chick anxiety-depression model utilizes socially raised chicks isolated at 4-6 days post-hatch. Chicks tested with conspecifics show low rates of vocalization throughout a 2 hour test session whereas chicks tested in isolation show high rates of distress vocalizations (DVocs) during the first 5 minutes (i.e., the anxiety-like phase) which decline over the next 20 minutes by 40-50% of the initial rate and remain steady throughout the remaining isolation period (i.e., depression-like phase). The reduced DVoc rates during this latter phase mirror the pattern seen in traditional behavioral despair depression models. Evidence that the 30-120 minute interval of isolation represents a depression-like phase comes from the ability of the tricyclic antidepressant imipramine to attenuate the decline in DVocs during this period (Sufka et al., 2006). Further research by this lab has demonstrated that all current FDA approved drugs for the treatment of depression have proven efficacious in the paradigm (Sufka et al., 2009; Warnick J., Wicks W., Sufka K., 2006). Antidepressant drugs that have screened positive include phenelzine, imipramine, citalopram, and maprotiline (Warnick et al., 2009, Feltenstein, M., Sufka, K., 2005).

Validation of the model has been examined by employing an endophenotypic mapping strategy as described by Josef van der Staay which involves matching, or reproducing, symptomology seen in the clinical setting, in the animal model. (van der Staay, 2006). Van der

Staay argues that the more clinical endophenotypes an animal model can reproduce the more valid the animal model is and better represents the actual disorder. The chick anxiety-depression simulation displays numerous homologies to the clinical features of depression. The model displays endophenotypes in a) etiological mediators of stress resilience via environmental enrichment (Kim E., Sufka K., 2011), b) alterations in biomarkers of stress (i.e., corticosterone) and depression (i.e., interleukin 6 and brain-derived neurotrophic factor (BDNF)) (Loria et al., 2013; Sufka et al., 2006; Warnick et al., 2009), c) cognitive biases in approach and avoidant behaviors (Hymel and Sufka, 2012; Salmeto et al., 2011), as well as d) pharmacological sensitivity in that it correctly screens FDA-approved pharmacotherapies (Warnick et al., 2006, 2009) and several novel compounds targeting non-monoamine-ergic systems, including ketamine, that have shown efficacy in clinical trials, as well as avoided two false positives from rodent screening models that failed clinical efficacy trials (Sufka et al., 2009).

Much of the endophenotypic validation work in the chick anxiety-depression model used a White Leghorn strain. However, recent research compared nine diverse genetic lines in the model and identified two strains in which one displayed stress vulnerability (Black Australorp) and the second stress resilience (Production Red) as measured by onset of behavioral despair (Hymel, et al., 2013). We argue that the Black Australorp strain, being faster to enter into behavioral despair (i.e. stress vulnerable), represents a similar endophenotype to TRD of severity of depressive symptoms. However, for the Black Australorp strain to be a true model of TRD, at least two classes of adequate dosing of antidepressants would have to fail to provide antidepressant effects in this strain in the chick anxiety-depression model. Therefore, the purpose of this study sought to explore whether these two lines display differential sensitivities to separate classes of FDA-approved antidepressant compounds by screening the tricyclic

antidepressant (TCA) imipramine, the selective norepinephrine reuptake inhibitor (SNRI) maprotiline and the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Given ketamine's clinical effectiveness in TRD, we also included it in our efficacy screening, noting this compound is used off-label for treatment resistant depression and depression with suicide ideation. The goal of this project was to determine whether the stress-vulnerable line displays a similar response pattern to these drug probes as patients diagnosed with TRD.

CHAPTER 3

METHODS

Cockerels (Ideal Poultry, Cameron, TX) were received into the laboratory at 1-2 days post hatch and housed in 34 x 57 x 40 cm cages with 9-15 chicks per cage (n = 12 is typical). Food and water are available ad libitum via gravity feeders. Daily maintenance that entails the replacement of tray liners and filling food and water gravity feeders is conducted during the hour that precedes the animal's dark cycle. Lights are operated on a 12:12 light dark cycle. Supplemental heating sources are provided to maintain appropriate housing temperatures in the range of 32 +/- 1 °C.

A six unit testing apparatus containing Plexiglas chambers (25 x 25 x 22 cm) surrounded by sound attenuating media is used to record separation-induced vocalizations aimed at modeling anxiety-like (0-5 minutes of social separation) and depression-like (30-120 minutes of social separation) patterns of responses [Figs. 2 & 3]. Each unit is lined with acoustical fiber media, illuminated by a 25-W light bulb, and ventilated by an 8-cm-diameter rotary fan (Model FP-108AX S1, Commonwealth Industrial Corp., Taipei, Taiwan). Miniature video cameras (Model PC60XP, SuperCircuits, Inc., Liberty Hill, Texas, USA) mounted in the sound-attenuating enclosures at floor level and routed through a multiplexor (Model PC47MC, SuperCircuits, Inc.) provided televised display of the chicks for behavioral observation. To record DVocs, microphones [Radio Shack Omnidirectional Model 33-3013 (modified for AC current)] are

mounted at the top of the Plexiglas chamber. These vocalizations are routed to a computer equipped with custom designed software for data collection.

Procedure

Separate hatches were used for each strain and dose response study. At days 5 and 6 post-hatch, animals were removed from their home cage in squads of six and placed within plastic transport container. To track subject assignment to various treatment conditions, chicks were marked using colored felt pens (i.e., six colors at two different body locations). Body weight was then measured for each chick to determine dosing and identify outliers (i.e., low body weight). The drug probes consisted of imipramine 5, 10, 15, and 20 mg/kg, fluoxetine 1, 5, and 10 mg/kg, maprotiline 2.5, 5, and 10 mg/kg, ketamine 5, 10, and 15 mg/kg or vehicle in a volume of 1 mL/kg body weight and were based on efficacy found previous studies. In the initial drug probe (i.e. the ketamine study), a separate group of vehicle-treated chicks served as non-isolated controls and were tested in the presence of two social companions and two mirrors positioned along the apparatus side-walls to simulate a social environment. These “social,” or non-isolated, birds display few distress vocalizations throughout the test session but were included as a control for each strain to highlight the stress manipulation. All compounds were administered intraperitoneal (IP) 15 minutes prior to isolation procedure. Following the 15 minute inject-to-test interval, squads were transported inside the lidded container to the adjacent testing room and each chick was placed into an individual testing unit. The program for recording distress vocalizations was started and allowed to run for 90 minutes where DVocs were recorded via custom designed computer software. Following the completion of each test session chicks were removed from the testing apparatus and returned to their home cage.

Records of the electronic files from the data collection program recording vocalizations were stored on the hard drive and backed up on a flash drive for data analysis. These procedures were approved by the University of Mississippi's IACUC (Protocol # 12-021)

Statistical Analysis

Data were screened for outliers before data analyses. This included excluding animals with body weights 15-20% below the mean weight of their shipping cohorts. Such animals are deemed to be developmentally delayed in motor activity and ability to distress call, and therefore may not respond adequately to the stressor. The total number of animals omitted based on this criteria equaled zero. Body weights between the two strains for the entire study were compared to rule out any extraneous variables and differences were found to be non-significant (Black Australorps mean weight equaled 48.8 and Production Red mean weight equaled 49.9) To highlight the change in distress vocalizations due to drug effects across the two anxiety-depression phases, DVoc data was converted to a rate per minute function. Thus, the anxiety phase was calculated as total DVocs during the first 3 min time block/3. For a more detailed analysis, the depression phase (i.e. 30-90 min time block) was divided into halves. The first 30 minutes (minutes 31-60), referred to as Dep. 1 and the second 30 minutes (minutes 61-90), referred to Dep. 2, and were calculated as the total DVocs during those individual 30 min time blocks/30. For analyses, DVoc rates for the anxiety and individual depression phases of the model were conducted using a one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) post-hoc tests. Vehicle treated animals that failed to show a stress effect or failed to enter into behavioral despair were omitted from analysis (n=8). This

criteria for omission was not applied to any of the test article animals as the behavioral effects of the selected test compounds had not been examined previously in these two strains and were unknown.

To highlight the stress effect Data were analyzed using one- (i.e., drug dose) and two-way (i.e., isolation test condition x test session length) ANOVAs. To highlight onset of behavioral despair between the two strains behavioral despair was calculated using the time point at which each chick's DVoc rate (i.e., counts/min) from its anxiety-like phase (first 3 min block) declined by 25, 50, 75 and 95% to the rate during its depression-like phase (30-90 min; Hymel et al. 2013; Kim E., Sufka K., 2011; Loria et al., 2013).

To highlight drug efficacy in the depression-like phase (30-90 min test period) was divided into four 15 minute quarters (30-45 min, 45-60 min, 60-75 min, and 75-90 min) to determine whether drug probes possessed antidepressant effects. DVoc rates for the four quarters of the depression-like phase were analyzed via 1-way ANOVAs followed by Fisher's LSD post-hoc tests. A dose that produced a statistically significant increase in DVoc rate compared to vehicle treated chicks was considered to possess antidepressant effects (i.e., attenuated behavioral despair in the model).

Sample sizes were $n = 11-12$. To protect against making a Type II error, a power analysis was included for all studies. Statistical significance for all analyses was considered at $p < .05$.

CHAPTER 4

RESULTS

Results highlighting the isolation stress effect and behavioral despair in Production Reds and Black Australorps are summarized in Fig. 1 panels a and b, respectively. In non-isolated chicks, DVoc rates were relatively low and remained stable throughout the test session. In contrast, isolated chicks displayed a robust increase in DVoc rates at the start of the test session that declined about 50% over the next 20-30 min and remained stable thereafter. A 2-way ANOVA revealed a significant main effect for Stress, $F(1,19) = 50.06$, $p < 0.0001$, a significant main effect for Test Session $F(29,551) = 3.82$, $p < 0.0001$ and a significant Stress x Test Session interaction term, $F(29,551) = 1.66$, $p = 0.018$. Simple effect analyses revealed a significant effect of Test Session in the Isolated group, $F(1,232) = 2.75$, $p < 0.0001$ but not in the non-isolated group ($p = \text{n.s.}$). This pattern in DVoc rates illustrates the two phases of the Anxiety-Depression model.

Like in Production Reds, DVoc rates in non-isolated Black Australorps chicks were relatively low and remained stable throughout the test session. Isolated chicks displayed a robust increase in DVoc rates at the start of the test session that declined about 50% over the next 10-20 min and remained stable thereafter. A 2-way ANOVA revealed a significant main effect for Stress, $F(1,21) = 65.11$, $p < 0.0001$ and a significant main effect for Test Session $F(29,609) = 3.84$, $p < 0.0001$. The Stress x Test Session interaction term failed to reach statistical significance ($p = 0.15$). A 1-way repeated measures ANOVA on isolated chicks revealed a

significant effect of Test Session, $F(1,290) = 2.64$, $p < 0.0001$). As before, this pattern in DVoc rates illustrates the two phases of the Anxiety-Depression model

Figure 1: Isolation Stress Effect

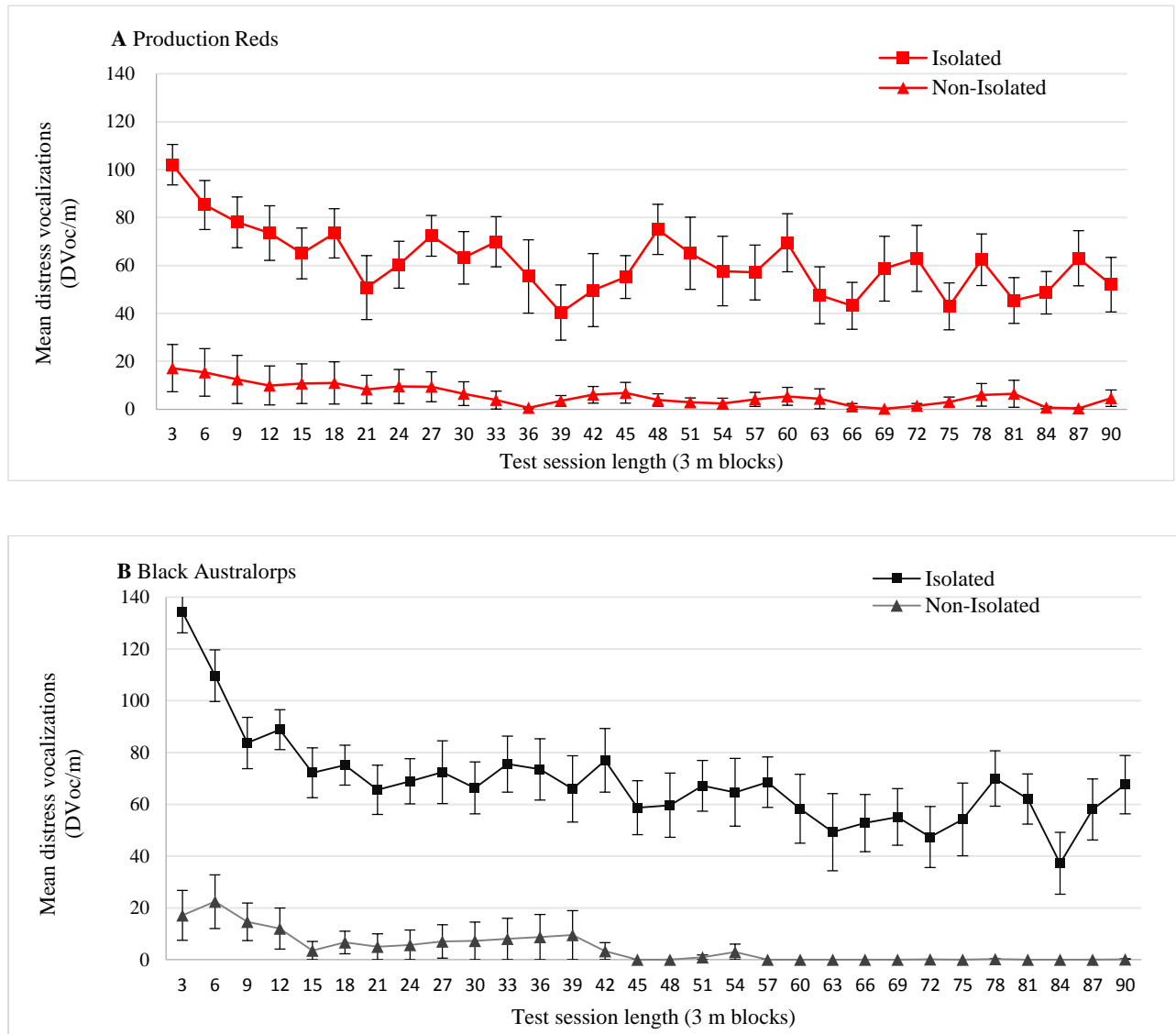


Figure 1. Mean distress vocalization (rate/m \pm SEM in 3 m blocks) across a 90-minute test session for isolated and non-isolated 5-6 day old vehicle-treated chicks in Production Red (A) and Black Australorp (B) lines. Sample sizes were $n = 9-12$.

Behavioral despair onset thresholds between strains for the vehicle-isolated groups from the dose response studies (pooled data) are summarized in Figure 2. Five cohorts (hatches)/strain were used for dose response studies (one dose response/drug except two dose responses for ketamine). Separate 1-way ANOVAs on DVoc rates in each phase (Anxiety-like phase: 0-5 min, Depression-like phase 30-90 min) of the model were conducted within each strain to determine cohort differences in base rates in vocalizations. These analyses revealed that 1 cohort in each strain displayed significantly different base DVoc rates from their respective cohorts. These 2 cohorts, which showed patterns of behavioral despair, were removed before calculating behavioral despair onset thresholds. In general, Black Australorps entered into behavioral despair at each threshold sooner than Production Reds. Consistent with these observations, 2-way ANOVA revealed a significant main effect for Strain $F(1, 84) = 6.59, p < 0.05$ and a significant main effect for behavioral despair onset Threshold, $F(3,252) = 77.15, p < 0.0001$. The Strain x Threshold interaction term was not statistically significant.

Figure 2: Behavioral Despair Onset between Strains

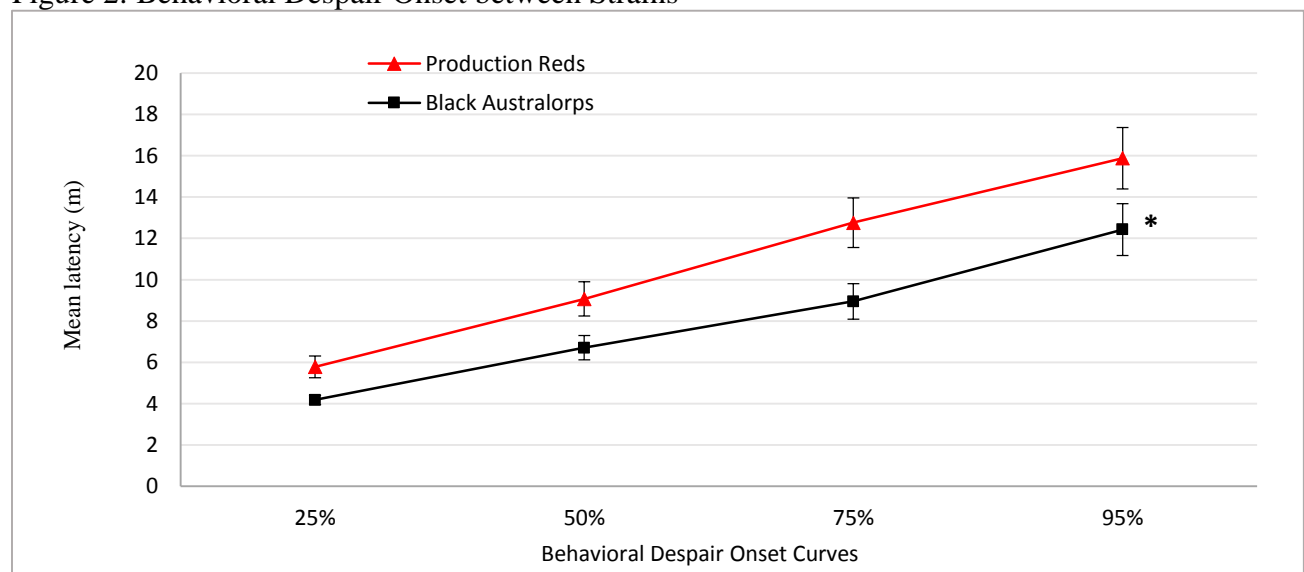


Fig. 2. Mean latencies (\pm SEM) to behavioral despair in Production Red and Black Australorp lines. * indicates significantly shorter latencies to behavioral despair (ANOVA main effect for strain, $p < 0.05$). Sample sizes were $n = 46-48$ from pooled cohorts (i.e., vehicle-isolated treated chicks from the individual drug probe studies).

The effects of test articles between the two strains across the isolation test session are summarized in figures 3 through 6. Imipramine possessed antidepressant activity in the early depression phase as indexed by a significant increase in DVoc rate in the Production Reds at the 5, 10, and 20 mg/kg doses $F(4, 54) = 1.90$, $p < 0.05$, power = .539. Imipramine showed no antidepressant effects in the late depression phase in Production Reds (Fig 3a). Unlike the Production Reds however, imipramine failed to alter DVoc rates in the Black Australorps at any dose tested in either the early or late depression phases F 's (4, 55) = .843 and 1.363, $p = \text{n.s.}$, power = .251 and .397 respectively. Fluoxetine failed to show antidepressant effects at any dose tested in either the early or late depression phases in the Production Reds F 's (3, 43) = 1.74 and .991, $p = \text{n.s.}$, power = .424 and .251 respectively (Fig 4a). Fluoxetine also failed to show antidepressant effects in either depression phase in the Black Australorps F 's (3, 42) = .433 and .407, $p = \text{n.s.}$, power = .129 and .124 respectively (Fig. 4b). Maprotiline produced an antidepressant effect at 2.5 and 5.0 mg/kg in both the early and late depression phases in Productions Reds F 's (3, 43) = 3.58 and 2.76, $p < 0.05$, power = .752 and .628 (Fig 5a) and at 2.5 mg/kg in both depression phases in the Black Australorps F 's (3, 43) = 1.629 and 3.498, $p < 0.05$, power = .397 and .741 respectively (Fig. 5b). Ketamine failed to affect DVoc rates in Production Reds in either depression phase F 's (3, 39) = .529 and .998, $p = \text{n.s.}$, power = .148 and .251 respectively (Fig. 6a) but did show significant antidepressant activity at the 5.0 and 10 mg/kg dose in Black Australorps F 's (3, 43) = 1.73 and 3.23, $p < 0.05$, power = .419 and .703 respectively (Fig. 6b). The high dose of ketamine, 15.0 mg/kg, did produce robust but brief ataxia in both strains two to three minutes after injection, but this effect was gone five minutes before isolation test. A follow-up study with ketamine at lower doses failed to see antidepressant activity in either strain.

Figure 3: Imipramine Drug Probes

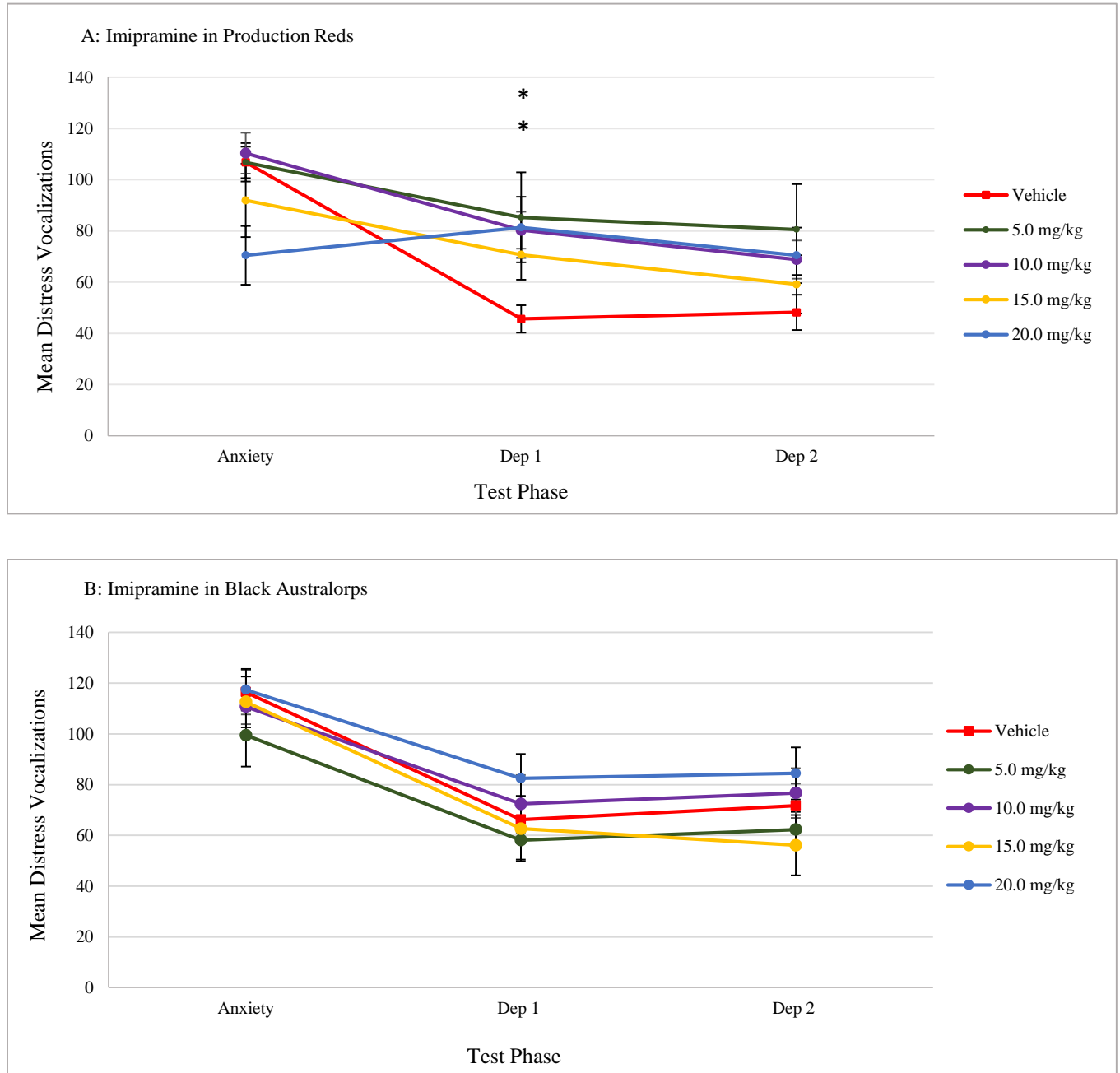


Fig 3. Mean distress vocalizations rate (+/- SEM) as a function of the three isolation phases per imipramine dose for Production Reds (3a) and Black Australorps (3b). * indicates a significant increase in DVocs in imipramine treated birds compared to vehicle treated birds per Fisher's LSD ($p < 0.05$; i.e. attenuation of behavioral despair). Sample sizes were $n = 11-12$.

Figure 4: Fluoxetine Drug Probes

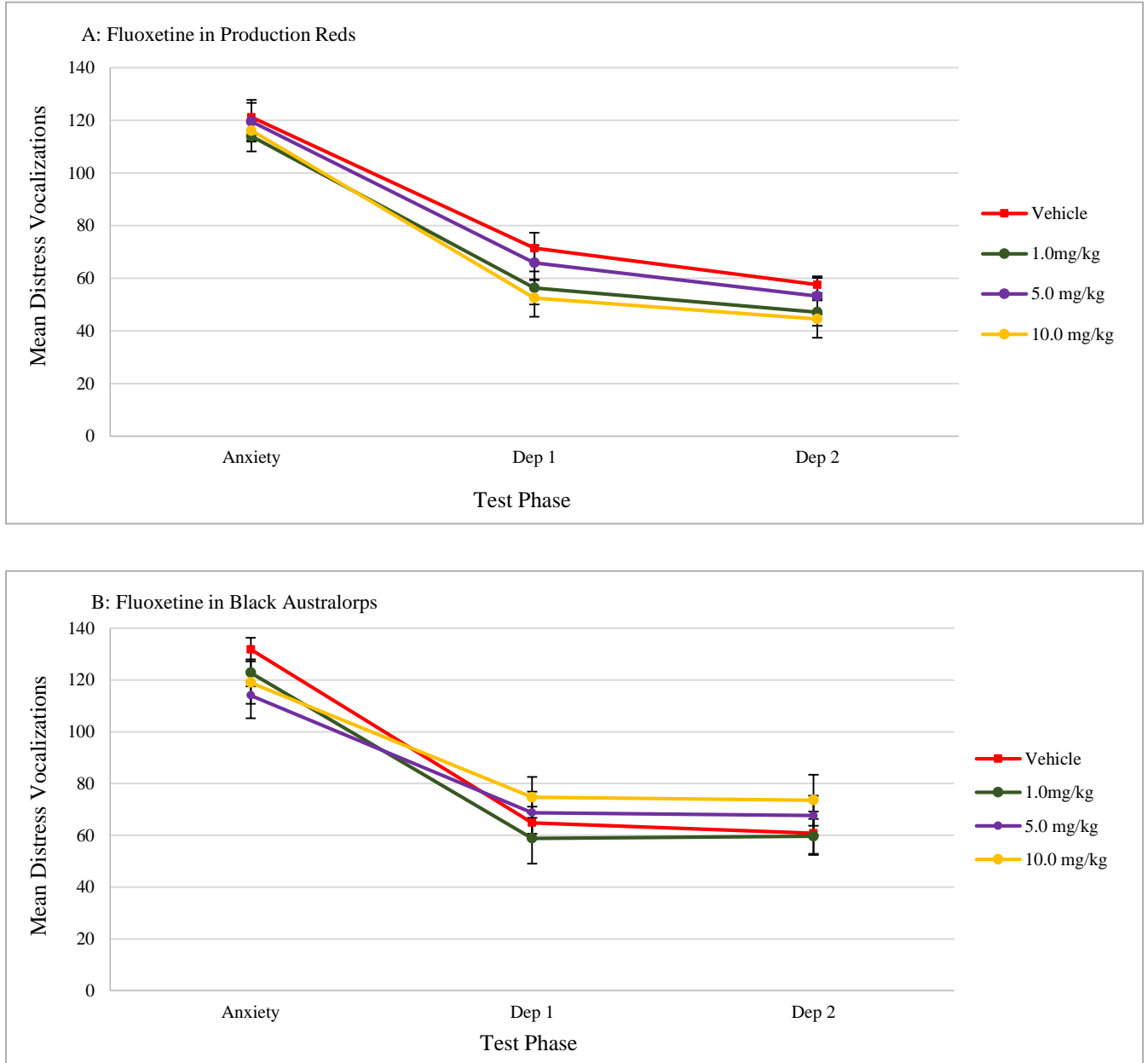


Fig 4. Mean distress vocalizations rate (+/- SEM) as a function of the three isolation phases per fluoxetine dose for Production Reds (4a) and Black Australorps (4b). * indicates a significant increase in DVocs in imipramine treated birds compared to vehicle treated birds per Fisher's LSD ($p < 0.05$; i.e. attenuation of behavioral despair). Sample sizes were $n = 11-12$.

Figure 5: Maprotiline Drug Probes

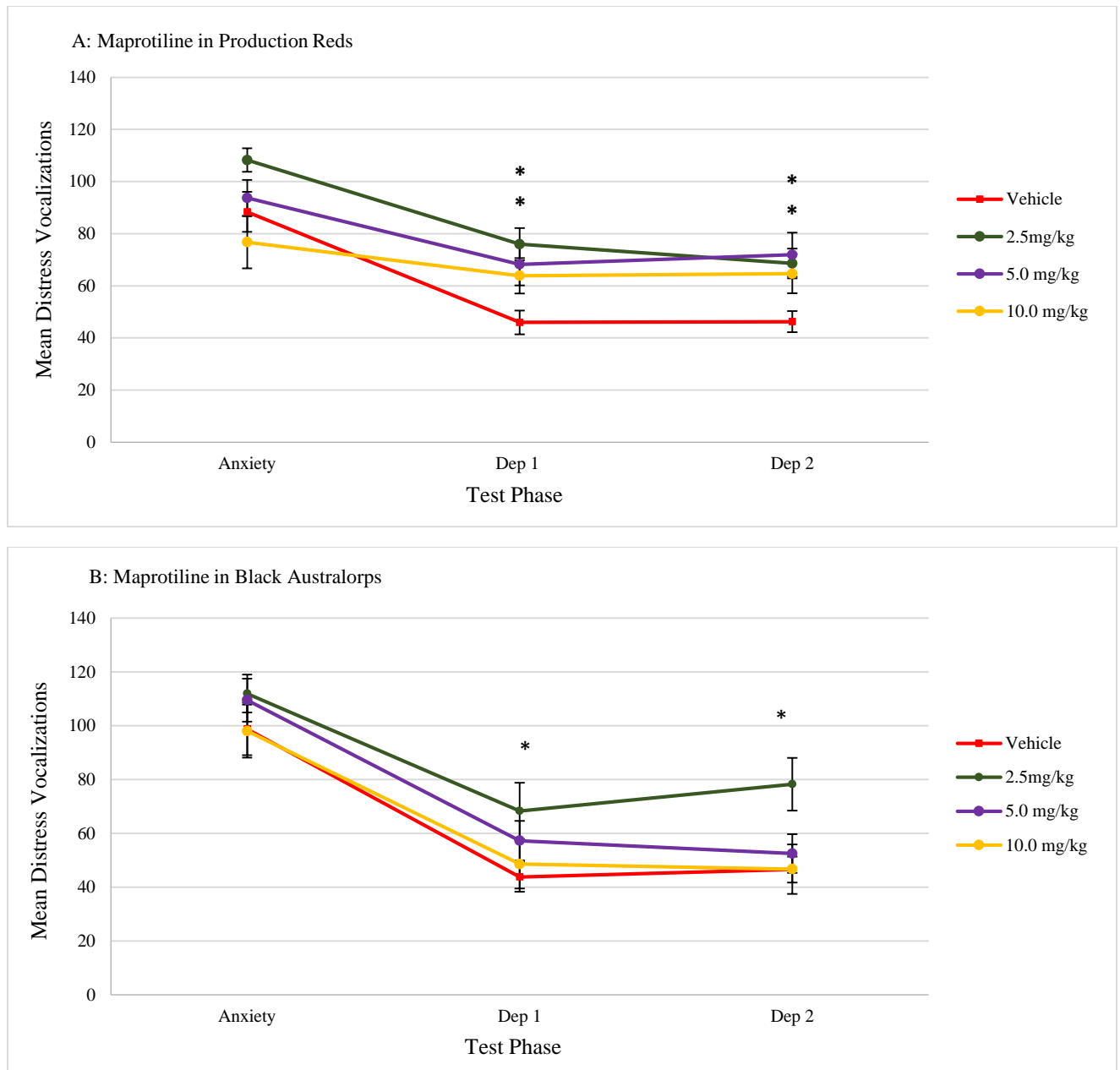


Fig 5. Mean distress vocalizations rate (+/- SEM) as a function of the three isolation phases per maprotiline dose for Production Reds (5a) and Black Australorps (5b). * indicates a significant increase in DVocs in imipramine treated birds compared to vehicle treated birds per Fisher's LSD ($p < 0.05$; i.e. attenuation of behavioral despair). Sample sizes were $n = 11-12$.

Figure 6: Ketamine Drug Probes

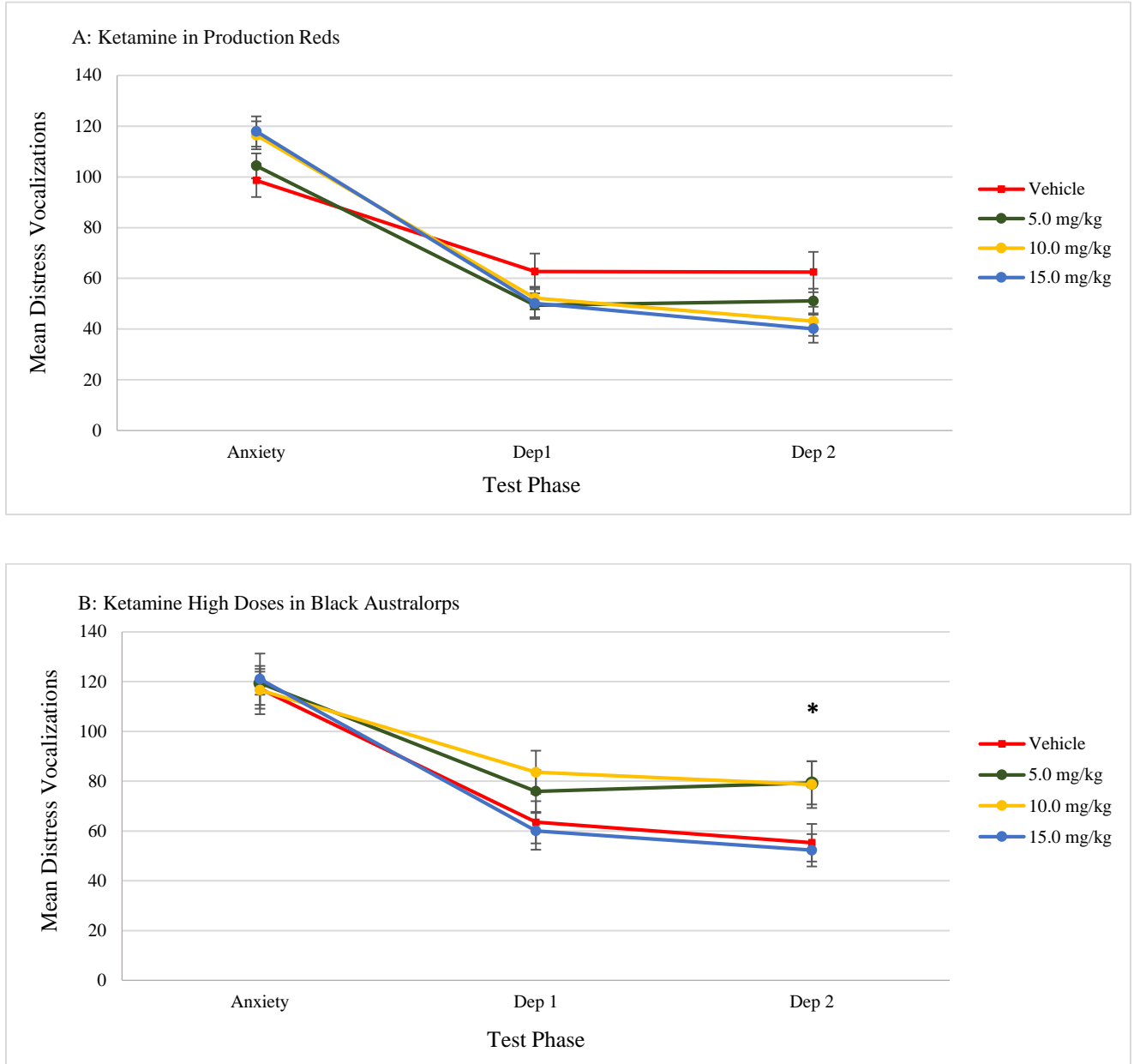


Fig 6. Mean distress vocalizations rate (+/- SEM) as a function of the three isolation phases per ketamine dose for Production Reds (6a) and Black Australorps (6b). * indicates a significant increase in DVocs in imipramine treated birds compared to vehicle treated birds per Fisher's LSD ($p < 0.05$; i.e. attenuation of behavioral despair). Sample sizes were $n = 11-12$

CHAPTER 5

DISCUSSION

The main goal of this project was to determine whether the stress-vulnerable Black Australorp genetic line would display a similar pharmacological response to various drug classes as patients diagnosed with TRD. A secondary goal of this research was to reproduce earlier findings highlighting the effects of social separation in the chick anxiety-depression model and the difference in the onset to behavioral despair between the two strains. One unexpected finding was the low power results for the studies. These results have lead us to increase our sample sizes in future studies from 12 to 18 in order to guard against making a Type II error and missing out on any significant antidepressant drug effects. A recent collaboration using sample sizes of 18 yielded power ranging from .80 to .99 (Lewellyn et al., 2013).

As seen in previous studies, non-isolated birds display few, if any DVocs during the entire test session, however, social separation produced an initial high rate of DVocs during the first five minutes, highlighting the anxiety-like phase, followed by a decline in DVoc rates by approximately 50% over the next 20-30 minutes and remained stable over the remainder of the test session. For the isolated birds, this last 60 minutes represents the depression-like phase. In the analysis of behavioral despair onset, the Black Australorps displayed shorter onset to behavioral despair (i.e. stress vulnerable) compared to Production Reds (i.e. stress resilient). These findings are consistent with earlier studies that characterize the sequential modeling of

anxiety- and depression-like phases as well as differentiating the Black Australorp and Production Red genetic lines as being stress vulnerable and resilient, respectively. Previous research has shown antidepressants attenuate the onset of behavioral despair in the chick social separation paradigm in the form of increased DVoc rates during the depression-like phase (Lehr E., et al., 1989; Sufka et al., 2006; Warnick et al., 2009). However, those studies utilized a White Leghorn strain and a summary of the current study with Production Reds and Black Australorps along with those of the White Leghorn strain is detailed in Table 1. The stress-resilient Production Red line showed sensitivity to two classes of FDA-approved antidepressants, the tricyclic imipramine and the tetracyclic maprotiline, but was insensitive to the SSRI fluoxetine and the NMDA antagonist ketamine. This was unexpected as we hypothesized this strain to be sensitive to all four compounds. This notion was based on previous studies where the White Leghorn strain showed sensitivity to all four compounds within the same dose range. The absence of a fluoxetine effect may reflect alterations in serotonin transporter functioning but not generally in serotonin transmission. As imipramine and maprotiline both exert their antidepressant effects on the norepinephrine transporter (NET), we hypothesize that the antidepressant effects seen with the Production Reds represents activity at this transporter site. However, we have yet to form a hypothesis as to why ketamine failed to show antidepressant effects in the Production Reds. Based on these findings do not believe this strain should be utilized as a preclinical screening beyond compounds that target the NET or post-synaptic serotonin receptors.

In contrast to the Production Reds, and White Leghorns, the stress-vulnerable Black Australorp line failed to show antidepressant sensitivity to the FDA approved antidepressants imipramine and fluoxetine across the broad dose range tested. However, this strain did show

antidepressant sensitivity to maprotiline at a limited dose range. Furthermore, the Black Australorp line showed antidepressant sensitivity to ketamine. The stress vulnerability along with the pattern of drug insensitivity and responsivity in the Black Australorp strain meets the clinical criteria of being treatment-resistant. To our knowledge, this unique pattern of drug response in a stress paradigm has yet been reported in the literature. We suggest this line is a lone model of TRD and useful for screening novel targets outside of the NMDA receptor for efficacy in TRD.

These initial findings suggest three possible directions for future studies, one that entails additional endophenotypic mapping, a second that explores central nervous system mechanisms that underlie ketamine's clinical benefits, and a third that entails identification of novel glutamatergic targets with clinical benefits for TRD.

TRD is often comorbid with other psychiatric disorders such as anxiety and substance abuse. The chick anxiety-depression model presents anxiety-, or panic-, like behavior represented by high rates of DVocs in the first several minutes of the separation stress procedure. Higher rates of DVocs during this anxiety-like phase could be indicative of a heightened panic-like state. In the clinical setting, anxiety patients suffer from cognitive bias in that they have more negative interpretations (i.e. more pessimism) of ambiguous stimuli or events. Recent research examining cognitive bias (i.e. more pessimism and/or less optimism) in chicks has shown differences between the black australorp and production red strains in measures of approach and avoidance to ambiguous stimuli in a straight alley maze (Hymel et al., 2013). However, these data were only recorded after chicks were isolated for a full 90 minute test session. Interestingly, the high dose of 20mg/kg Imipramine showed anxiolytic activity in the Production Red strain, $p < .05$, a finding consistent with the clinical picture of Imipramine, but failed to show such a response in the Black Australorp strain. Future research should examine

cognitive bias differences immediately following the anxiety-like phase of the isolation stressor and behavioral responses to FDA approved anxiolytics between the two strains.

Ketamine's clinical benefits are thought to be mediated through activation of post-synaptic glutamatergic AMPA receptors. AMPA receptor activation and potentiation leads to the activation of intracellular mammalian target of rapamycin (mTOR) pathway which triggers a cascade of process that ultimately increase production of BDNF (Li N., et al., 2010, Li X., et al., 2001, Maeng S., et al., 2008). Research in rodent models of depression have shown that blockading either the AMPA receptor site or preventing mTOR activation prevents ketamine's antidepressant effects (Maeng S., et al., 2008, Li N., et al., 2010). Exploration of these mechanisms would provide further validation of the proposed Neurotropic Theory of depression as well as lend further validation to the model itself.

However, over stimulation of AMPA receptors can lead to excitotoxicity and cell death (Frandsen A., et al., 1989). Therefore, finding alternate glutamatergic targets that can modulate synaptic glutamate levels and thus indirectly stimulate AMPA receptors could be a third avenue of future research. Metabotropic glutamate receptors could be a viable target for testing, and a number of preclinical rodent screens have shown that modulation of metabotropic glutamate receptors results in antidepressant effects (Palucha A., et al., 2004, Palucha A., et al., 2005, Palucha A., et al., 2007, Palucha A., et al., 2010, Belozertseva I., et al., 2006, Chaki, S., et al., 2004,).

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